Relationship between plasma iron concentration and gastric pH in captive adult bottlenose dolphins (*Tursiops truncatus*)

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Objective—To determine the correlation between plasma iron concentrations and gastric pH in a population of captive Atlantic bottlenose dolphins (*Tursiops truncatus*).

Animals—6 adult female dolphins that ranged from 16 to 30 years of age.

Procedures—Blood and gastric samples were collected from each dolphin to allow measurement of plasma iron concentrations and gastric pH, respectively. Samples were collected each month for 12 months.

Results—Within each dolphin, plasma iron concentrations and gastric pH did not differ significantly over time. There was a strong negative correlation ($r = -0.85$) between plasma iron concentration and gastric pH, which suggested that dolphins with a lower gastric pH had a higher plasma iron concentration.

Conclusions and Clinical Relevance—Analysis of results reported here suggested that gastric pH may play an important role in iron absorption in dolphins. (*Am J Vet Res* 2008;69:900–903)

Iron is an important mineral that serves many functions in the body, including oxygen transport and oxidative metabolism.[1] However, iron can also act as a toxin. Hemosiderosis, a pathologic condition that results when excessive iron is stored in tissues, has been described in birds, primates, rhinoceri, and other zoologic species.[2,3] Hemosiderosis has also been described in 2 dolphins (1 captive and 1 wild).[4,5] In a captive bottlenose dolphin (*Tursiops truncatus*), hemosiderosis and acute lead toxicosis were diagnosed during necropsy.[4] Necropsy revealed that the wild dolphin, a stranded pregnant Risso's dolphin (*Grampus griseus*), had disseminated toxoplasmosis and hemosiderosis.[5] It is highly likely that hemosiderosis is more common in dolphins than has been reported, and research to describe the cause of this disease is needed.

Although the exact mechanisms associated with development of hemosiderosis in susceptible species are not known, dietary deficiencies, excessive supplementation of iron, infectious diseases, toxins, corticosteroids, and congenital factors have all been suggested as possible factors.[2,3,6,7] In captive birds and primates, hemosiderosis is frequently attributed to excessive dietary iron.[2] The authors have gathered data on dietary iron provided to a captive population of dolphins. In our experience, the amount provided was higher than that expected for humans. Variation in iron concentrations among the species of fish being fed to the dolphins was also detected. Hemosiderosis has been diagnosed in the population of dolphins from which we accumulated those data, and the dolphins had variations in plasma iron concentrations. Differences in the plasma iron concentrations among dolphins with a similar diet suggested that the discrepancy in plasma iron concentrations was associated with factors other than the diet.

A number of factors can affect the absorption of iron, including gastric acidity, plant materials, other divalent cations, and infectious diseases.[1,2,8,9] One particular factor that receives a great deal of interest in human medicine is gastric pH. In an acidic pH, chelated cations become ionized, which increases their rate of absorption.[8,9] In veterinary medicine, this is commonly reported with lead and zinc toxicosis in avian patients that have ingested heavy metals.

The purpose of the study reported here was to determine whether gastric pH was correlated with plasma iron concentrations in a population of captive Atlantic bottlenose dolphins. The specific hypothesis tested was that gastric pH values would be inversely correlated with plasma iron concentrations in captive dolphins. This information will be of value for determining the epidemiologic aspects of hemosiderosis in captive dolphin populations.

Materials and Methods

Animals—Six adult female dolphins that ranged from 16 to 30 years of age were used in the study. None of the dolphins had hemosiderosis. All of the dolphins

Received June 25, 2007.
Accepted November 16, 2007.
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Supported by the Institute for Marine Mammal Studies.
The authors thank Lana Courtney for assistance with sample processing.
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Table 1—Gastric pH values in samples obtained monthly for a 12-month period from each of 6 captive adult bottlenose dolphins (*Tursiops truncatus*).

Dolphin	Range	Mean ± SD	Median	25th to 75th percentiles
1	1.7–5.5	3.7 ± 1.1	—	—
2	1.4–2.2	1.7 ± 0.3	—	—
3	1.3–1.9	1.6 ± 0.2	—	—
4	1.5–6.5	—	1.7	1.5–2.0
5	1.5–3.9	—	1.8	1.6–3.0
6	1.3–4.2	—	1.8	1.6–2.7

Normally distributed data were reported as mean ± SD, and data that were not normally distributed were reported as median and 25th to 75th percentiles. — = Not applicable.

in the study were long-term captives housed in 3 pools at the Marine Life Oceanarium in Gulfport, Miss. The study was conducted in accordance with regulations specified by the Louisiana State University Institutional Animal Care and Use Committee.

Procedures—A longitudinal study was conducted from March 2004 through February 2005. Trainers collected blood and gastric samples monthly from each dolphin.

Gastric samples were collected in the morning before dolphins were fed. A 1.9-cm gastric feeding tube was passed through the oral cavity and into the stomach. Gastric contents (3 to 8 mL) were collected by use of a negative-pressure siphon technique. Contents were immediately examined at poolside. The general appearance of the gastric sample was evaluated, and pH of the solution was measured in triplicate by use of a pH meter[a]; the pH meter was calibrated by use of a standard solution (pH, 4.0) before testing of each sample. The arithmetic mean of the gastric pH was used for analysis.

The venipuncture site was cleansed with 70% ethyl alcohol prior to blood collection. A blood sample (6 mL) was collected from the superficial fluke vein of each dolphin by use of a 21-gauge or 23-gauge butterfly catheter. Blood samples were placed directly into lithium heparin anticoagulant tubes. Blood samples were centrifuged (4,000 $\times$ *g* for 5 minutes) within 2 hours after collection. Plasma was pipetted into sterile cryovials. Samples were examined for hemolysis and discarded when the plasma was grossly discolored. Plasma samples were transported on wet ice to Louisiana State University within 6 hours after blood collection. Plasma iron concentrations were determined by use of furnace atomic absorption spectrophotometry[b] on the same day that they were obtained. The machine was calibrated by use of a commercial standard[c] prior to processing of samples.

Statistical analysis—Distribution of samples was evaluated separately for each dolphin and on the basis of each dolphin's pool location. The Shapiro-Wilk test was used to evaluate distribution of the data. Mean, SD, median, 25th to 75th percentiles, and range were determined for plasma iron concentrations and gastric pH. For normally distributed data, the mean and SD were reported, and a 95% confidence interval was calculated. For data that were not normally distributed, the median and 25th to 75th percentiles were reported. Data that were not normally distributed were logarithmically transformed for analysis. A general linear model for

Table 2—Plasma iron concentrations in samples obtained monthly for a 12-month period from each of 6 captive adult bottlenose dolphins.

Dolphin	Range	Mean ± SD	95% CI
1	3.3–4.5	3.8 ± 0.4	3.5–4.0
2	7.0–12.9	9.6 ± 1.9	8.4–10.9
3	9.6–22.8	14.7 ± 3.6	12.3–17.1
4	3.9–6.2	4.8 ± 0.9	4.2–5.4
5	6.4–11.4	8.6 ± 1.3	7.5–9.3
6	1.9–7.9	4.6 ± 1.6	3.6–5.7

Values reported are µg/mL.
CI = Confidence interval.

repeated measures was used to determine differences in gastric pH or plasma iron concentrations over time within each dolphin or among pool locations (between dolphins). When there were no within- or between-dolphin differences, a reference range was established for gastric pH and plasma iron concentrations. The Pearson correlation coefficient was used to assess the relationship between mean gastric pH and plasma iron concentrations. A commercially available statistical program[d] was used to perform statistical analyses. A value of $\alpha \leq 0.05$ was used to determine significance.

Results

Gastric pH ($F = 0.4$; $P = 0.5$) or plasma iron concentrations ($F = 2.7$; $P = 0.2$) did not differ significantly over time within each dolphin. This indicated that within each dolphin, gastric pH and plasma iron concentrations did not vary significantly from month to month. There was also no significant difference in gastric pH ($F = 2.3$; $P = 0.25$) or plasma iron concentrations ($F = 0.25$; $P = 0.8$) among the pool locations. Because there was no significant difference between these samples, a reference range was calculated for each dolphin **(Tables 1 and 2)**. A strong negative correlation ($r = -0.85$; $P = 0.05$) was detected between gastric pH and plasma iron concentrations **(Figure 1)**.

Discussion

Hemosiderosis is an insidious disease. Animals with increases in blood and tissue concentrations of iron may have an increased susceptibility to bacterial infections and organ dysfunction. In captive animals, hemosiderosis is unlikely to be diagnosed, unless routine screening is performed. For captive dolphins, a diagnosis of hemosiderosis is most often made during necropsy.[4] An antemortem diagnosis can be made by

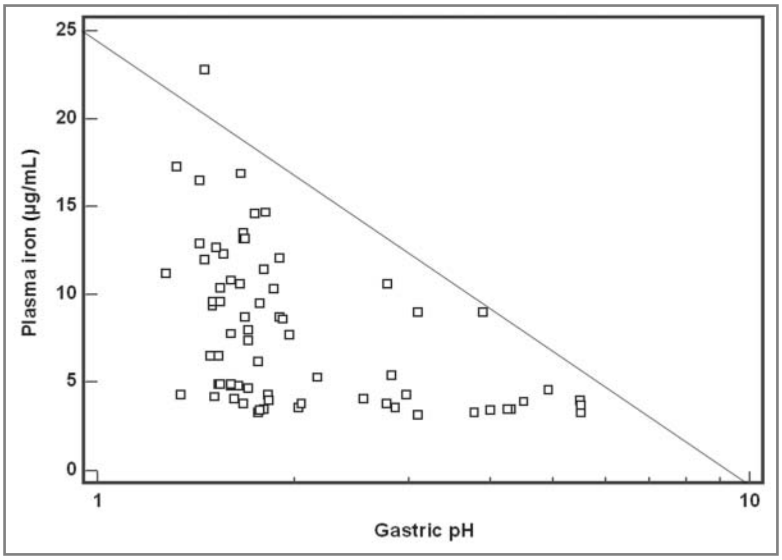

Figure 1—Scatterplot of plasma iron concentrations and gastric pH values in samples obtained each month during a 12-month period from 6 captive adult bottlenose dolphins (*Tursiops truncatus*). A strong negative correlation ($r = -0.85$; $P = 0.05$) was detected between gastric pH and plasma iron concentrations.

examination of liver biopsy specimens, but this is not routinely done. To reduce the likelihood of encountering unexpected deaths or obtaining the diagnosis late in the course of the disease, improved methods of diagnosis and identification of potential risk factors associated with the cause of hemosiderosis are needed.

A potentially noninvasive method for assessing iron storage in the body is to measure circulating iron concentrations in the blood. Unfortunately, in the experience of one of the authors (PJ), concentrations of cations do not necessarily correlate with tissue concentrations. However, in children with leukemia, serum iron concentrations are highest in patients with the highest iron concentrations in the liver.[10] This type of association may provide clinicians with some predictive capability when interpreting hematologic results. In the study reported here, there were 2 distinct groups of dolphins, as determined on the basis of the reference range and 95% confidence interval calculated for the plasma iron concentration for each dolphin. Plasma iron concentrations for 3 dolphins were higher than those for the other 3 dolphins. Whether the dolphins in the study would also have variations in hepatic iron concentrations is unknown but should be determined.

Hemosiderosis in many of the birds and mammals in zoologic parks has been attributed to excessive amounts of iron in the diet.[2] In the experience of members of our laboratory group with dolphins at the same oceanarium, the discrepancy identified in plasma iron concentrations among dolphins could not be directly attributed to diet. Results of the study reported here in which gastric pH was negatively correlated with plasma iron concentrations suggested that physiologic variation among dolphins may affect circulating iron concentrations. This is further reinforced by the fact that the plasma iron concentrations did not differ within each dolphin over time. Because cations, such as iron, become ionized in acidic solutions, it could be expected that more iron would be absorbed through the gastrointestinal tract in dolphins with lower gastric pH values. Again, without tissue samples to confirm a diagnosis of hemosiderosis, we can only speculate that dolphins with high plasma iron concentrations would also have high tissue concentrations of iron.[10]

Gastric acid secretion in mammals fluctuates during the day and is influenced by diet, stress, and infectious diseases.[8,11,12] When there is a reduction in gastric acid secretions, iron absorption can also be reduced.[11,12] *Helicobacter pylori*, an important bacterial pathogen of the gastrointestinal tract in humans, can reduce gastric acid secretions. In children with *H pylori* infections, iron deficiency anemia can develop.[12] Gastric acid secretions in dolphins can fluctuate during the day.[13] In another study[13] in which investigators evaluated serial gastric pH values in 3 of the same dolphins used in the study reported here, gastric pH values were lowest prior to when the dolphins were initially fed in the morning. Gastric acid concentrations can increase immediately prior to feeding. This is a common response when a human or other animal recognizes that a meal is imminent. Whether the gastric acid content in samples from the dolphins varied because of differences in behavior, infectious disease status, or sampling technique could not be concluded from our study. However, by collecting samples from the dolphins at the same time of day during a 12-month period, we were able to develop a reference range for gastric pH for each dolphin, and the results suggested that there was minimal variability within each dolphin.

It was apparent from analysis of the data that the mean or median gastric pH for most of these dolphins was < 2.0 (Table 1). Although these values appear similar, it is also important to recognize that the SD, 25th to 75th percentiles, and ranges for gastric pH values revealed some degree of variability among dolphins. This was especially true for 3 dolphins, which were also the dolphins with the lowest plasma iron concentrations. It is also important to consider that measures of gastric pH are based on a logarithmic scale, which means that apparently small differences are larger than their numeric value might imply. On the basis of the study reported here, it is not possible to determine how such small incremental changes in gastric pH would affect iron absorption. In humans, differences in gastric pH as great as 1.5 have a major effect on iron absorption.[14] Whether similar or smaller differences in gastric pH in dolphins would also affect iron absorption is not known but should be evaluated.

Although additional research is needed to fully elucidate the role gastric pH has on iron absorption in captive dolphins, clinicians who care for these animals may consider use of antacid treatments to potentially counteract the effects of low gastric pH in situations where hemosiderosis is a concern. Cimetidine, a histamine type 2–receptor antagonist, can effectively reduce iron absorption in humans by decreasing gastric acid secretion.[11] The reduction in iron absorption is a dose-dependent event. In addition, antacids also reduce iron absorption in human patients.[11] Cimetidine and antacids are currently used in the treatment of captive dolphins with gastric ulcers, and they may also exert a secondary affect by minimizing iron absorption. In animals with low to moderate iron concentrations, long-term use of cimetidine and antacids could be detrimental and result in iron deficiency anemia.

Analysis of results of the study reported here suggested that lower gastric pH was highly correlated with higher plasma iron concentrations in dolphins. This information represents initial attempts to elucidate a physiologic factor that may affect iron absorption in dolphins. Additional studies are required to characterize the effects that diet, training, and captivity have on gastric acid concentrations in dolphins.

a. Model PH220, Extech Instrumentation Corp, Waltham, Mass.
b. Perkin-Elmer 800, Perkin-Elmer Corp, Wellesley, Mass.
c. Seronorm, Accurate Chemical and Scientific Corp, Westbury NY.
d. SPSS, version 11.0, SPSS Inc, Chicago, Ill.

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